

## A De Novo Mutation of *STK11* Gene in a Chinese Patient with Peutz–Jeghers Syndrome

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**Abstract** Peutz–Jeghers syndrome (PJS) is an autosomal-dominant inherited disorder characterized by mucocutaneous pigmentation, hamartomatous polyposis of the gastrointestinal tract, and an increased risk for the development of both gastrointestinal and extraintestinal malignancies. Germline mutation of the *STK11* gene, which encodes a serine-threonine kinase, is responsible for PJS. We collected blood samples from a Chinese PJS family consisting of a total of four individuals (one male and three females) including one PJS patient. The whole coding region of *STK11* was amplified by polymerase chain reaction and products analyzed by direct sequencing. Molecular analysis of the *STK11* gene in this case of PJS revealed a substitution of thymine 217 for adenine (C.217T > A) in exon 1, resulting in a change of codon 73 from cysteine to serine (C73S). The point mutation was not found in normal individuals in this PJS family or in 100 control individuals. The results presented here enlarge the spectrum of mutations of the *STK11* gene by identifying a de novo mutation in a PJS patient and further support the hypothesis that *STK11* mutations are disease-causing mutations for PJS.

**Keywords** Peutz–Jeghers syndrome · *STK11* · Mutation analysis · DNA sequencing

### Introduction

Peutz–Jeghers syndrome (PJS; OMIM 175200) is a rare cancer predisposition, which is characterized by the presence of hamartomatous polyposis and mucocutaneous pigmentation. The first report describing Peutz–Jeghers syndrome (PJS) was probably by a London surgeon, Jonathan Hutchinson, in 1896. The association of mucocutaneous pigmentation with multiple gastrointestinal polyps was first described in 1921 by Peutz who reported this association in several members of a family. In 1949, Jeghers et al. [1] established the syndrome as an entity appearing to be inherited as an autosomal-dominant condition. The main clinical symptoms of the syndrome include abdominal pain, rectal blood loss, anemia, small bowel obstruction, and intussusception leading to a high laparotomy rate. Patients with PJS are at an increased risk of developing gastrointestinal cancer and extraintestinal neoplasms involving organs such as the ovaries, testes, breasts, pancreas, lungs, or uterine cervix [2]. The incidence of the syndrome is estimated to be between 1 in 8,300 and 1 in 120,000 [3].

In 1997, Hemminki et al. [4] utilized comparative genomic hybridization of hamartoma from a single PJS patient to detect loss of the telomeric region of chromosome 19p13.3, and subsequently mutations in a gene encoding a serine-threonine kinase, *STK11* (other aliases *LKB1*, OMIM 602216) have been found in the majority of the patients ([5], [6]). *STK11* is a known tumor suppressor and several studies reported that somatic mutations have been found at a low frequency in sporadic tumors of the colon, stomach, ovary, testes and lungs [7–10]. *STK11* consists of nine coding exons with a 433-amino acid coding sequence and one non-coding exon 10. Codons 49–309 encode the catalytic kinase domain. Most of different

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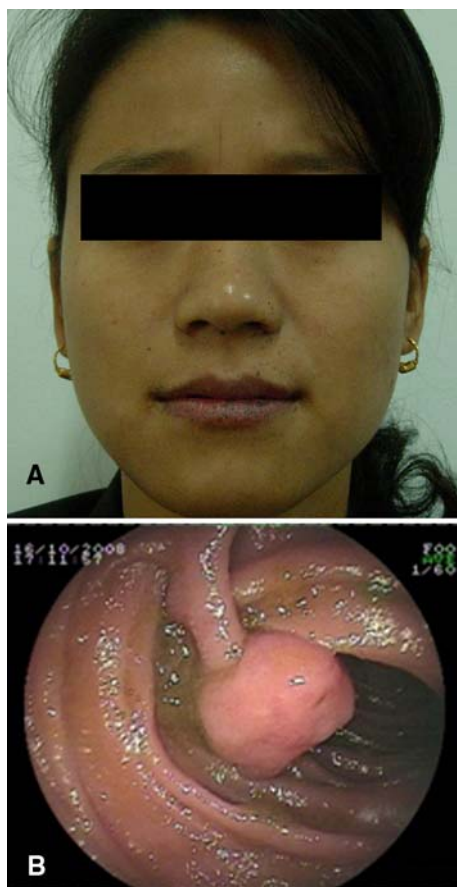
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mutations in the *STK11* gene which have been reported are small insertions/deletions or missense/nonsense mutations. Here, we report a novel *STK11* mutation in a Chinese PJS patient.

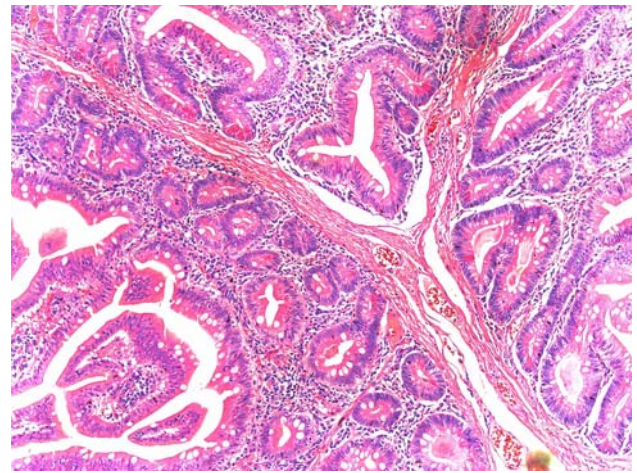
## Materials and Methods

### Patient and Sample Collection

The proband was a 25-year-old Chinese female. Her past medical history revealed that the mucocutaneous pigmentation (Fig. 1a) had appeared for 20 years and she had developed abdominal pain at the age of 12 years. Endoscopy had been performed when she was 25 years old and showed gastrointestinal tract multiple polypi (Fig. 1b). Some polypi with low-grade dysplasia were removed by polypectomy under endoscopy. The polypi in the jejunum were surgically removed and confirmed as hamartomatous polyp (Fig. 2). Her relatives had no known medical conditions, including mucocutaneous pigmentation or



**Fig. 1** a Subtle perioral melanin pigmentation spots of the lips. b Double-balloon enteroscopy identified the jejunum polyp



**Fig. 2** Pathologic findings of the Peutz–Jeghers polyp. The polyp of jejunum shows Hamartomatous polyp of the jejunum (Hematoxylin-eosin stain, 100 $\times$ )

malignancies. Her normal sister and parents confirmed no polyps by endoscopic examination.

After obtaining informed consent, blood samples were collected from four family members including the patient.

### Genomic DNA Isolation

The genomic DNA was isolated from peripheral blood leukocytes using a Kit (Promega, USA) according to the manufacturer's instructions.

### DNA Sequencing

The *STK11* gene was amplified via PCR, using the appropriate primers as according to the literature [11]. PCR was performed in 50  $\mu$ l reaction volume containing 50 ng of genomic DNA, 0.3 mM dNTPs, 0.3  $\mu$ M of each primer, 3.0 mM MgCl<sub>2</sub> and 0.3 units of Hotstar<sup>®</sup>Taq DNA polymerase (Qiagen). The PCR conditions were: Hotstar<sup>®</sup>Taq activation at 95°C for 12 min, followed by 40 cycles, each having denaturation at 94°C for 40 s, annealing at 58°C for 60 s and extension at 72°C for 55 s, except that in the first 10 cycles the annealing temperature decreased from 63 to 58°C by 0.5°C per cycle, and the final extension was 72°C for 10 min. After the amplification, PCR products were purified using a QIA quick PCR Purification Kit (Qiagen) and sequenced using ABI PRISM<sup>®</sup> 3700 automated sequencer (Applied Biosystems). Sequence comparisons and analysis were performed using Phred-Phrap-Consed Version 12.0 program. Samples from 100 unrelated controls were sequenced for missense to exclude the possibility that these are polymorphism in the *STK11* gene.

## Results

Direct sequencing analysis of the DNA from the proband showed a substitution of thymine 217 for adenine (C.217T > A) in exon 1 of the *STK11* gene, resulted in a heterozygous missense mutation of cysteine(C) to serine(S) substitution at the 73rd codon. This mutation was absent in her family members and 100 control chromosomes (Fig. 3).

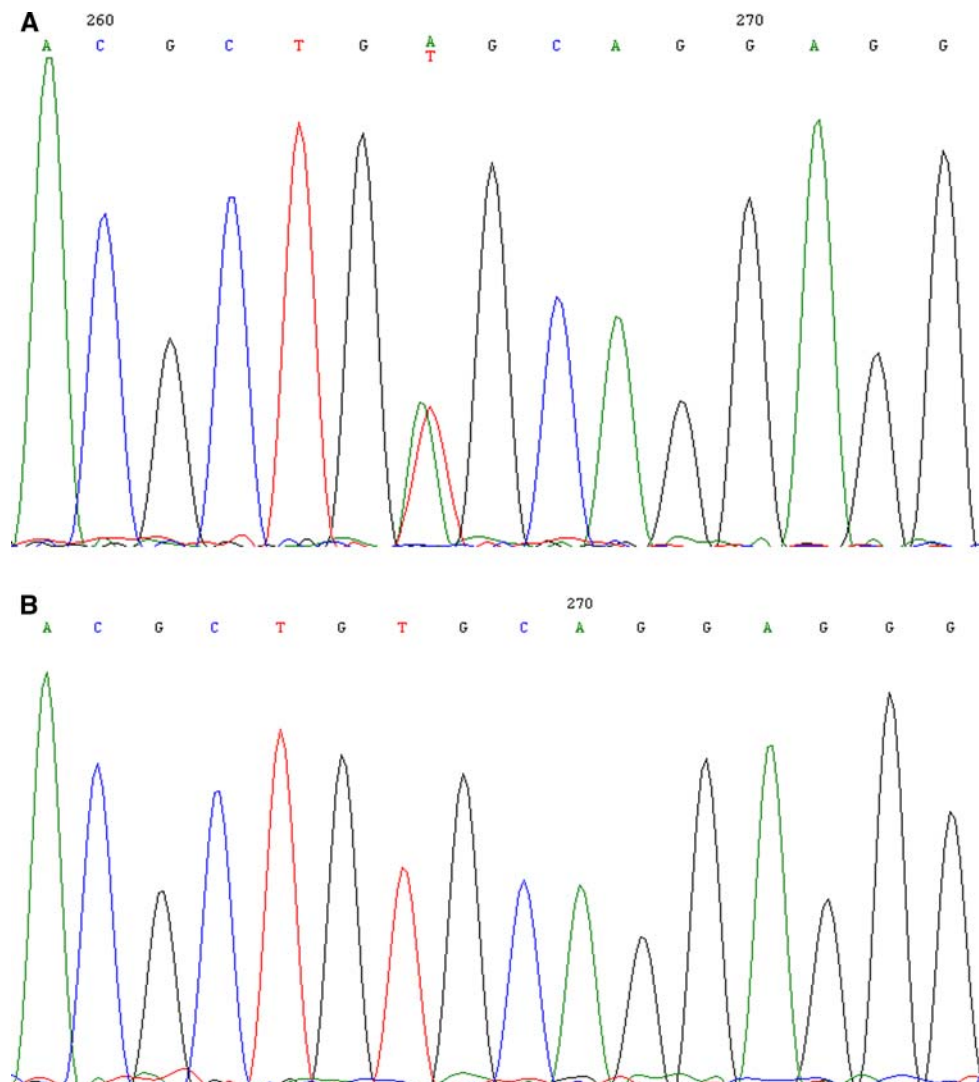
## Discussion

PJS has been associated with germline mutations in the *STK11/LKB1* gene [5, 11–14]. Initial reports suggested the presence of sequence alterations in *STK11/LKB1* in most PJS patients. However, recent studies suggest that *STK11/LKB1* mutations only account for 40–60% of families with PJS [13]. This suggests the presence of significant genetic

heterogeneity for PJS and the involvement of other loci in this syndrome, and another study suggest a second locus is suspected on chromosome 19q13.4 in a minority of families [15].

The *STK11/LKB1* protein is ubiquitously expressed in all human fetal and adult tissues [16]. Homozygous deletion of *STK11/LKB1* in mice leads to embryonic lethality at midgestation (E11.0), indicating that *STK11/LKB1* plays an important role in embryogenesis [17, 18]. Interestingly, most of the *STK11/LKB1*<sup>(+/-)</sup> mice developed intestinal polyps by the age of 45 weeks, identical to those observed in patients with PJS [10, 19]. *STK11/LKB1* protein is mainly comprised of three major domains: the N-terminal non-catalytic domain containing the nuclear localization signal, the catalytic kinase domain important for ATP binding, and the carboxyterminal non-catalytic regulatory domain containing prenylation motif (CAAX box). Codons 49–309 encode the catalytic kinase domain. The C-terminal non-catalytic region of the *STK11/LKB1*

**Fig. 3 a** Identification of the *STK11* gene mutation: direct sequencing of the proband showed a heterozygous missense mutation of C.217T > A in exon 1 of the *STK11* gene, resulting in a heterozygous missense mutation of C73S. **b** The proband's family members did not have the mutation



**Fig. 4** Amino acid alignment of various species around the one missense mutation: human *STK11* amino acid residues 62–112 are shown aligned to known *STK11* orthologues. Residues C, at 73, is conserved in all species compared in the figure. Absolutely conserved and partly conserved amino acid residues among all available sequences are shown with red and yellow backgrounds, respectively. All data were cited from GenBank. (Color figure online)

		73 ↓	
NP_ <i>Homo sapiens</i> (human)	62	EVEVLDSEITLCEERAVKILKMKKLERIPNGEAVKKETIQARRRREKNIQ	112
XP_ <i>Pan troglodytes</i> (chimpanzee)	62	EVEVLDSEITLCEERAVKILKMKKLERIPNGEAVKKETIQARRRREKNIQ	112
XP_ <i>Canis lupus familiaris</i> (dog)	62	EVEVLDSEITLCEERAVKILKMKKLERIPNGEAVKKETIQARRRREKNIQ	112
XP_ <i>Bos taurus</i> (cattle)	62	EVEVLDSEITLCEERAVKILKMKKLERIPNGEAVKKETIQARRRREKNIQ	112
NP_ <i>Mus musculus</i> (house mouse)	62	EVEVLDSEITLCEERAVKILKMKKLERIPNGEAVKKETIQARRRREKNIQ	112
XP_ <i>Rattus norvegicus</i> (Norway rat)	62	EVEVLDSEITLCEERAVKILKMKKLERIPNGEAVKKETIQARRRREKNIQ	112
NP_ <i>Gallus gallus</i> (chicken)	62	EVEVLDSEITLCEERAVKILKMKKLERIPNGEAVKKETIQARRRREKNIQ	112
NP_ <i>Danio rerio</i> (zebrafish)	61	EVEVLDSEITLCEERAVKILKMKKLERIPNGEAVKKETIQARRRREKNIQ	111
NP_ <i>Drosophila melanogaster</i> (fruit fly)	185	EVEVLDSEITLCEERAVKILKMKKLERIPNGEAVKKETIQARRRREKNIQ	235

protein is encoded by exon 8 and 9 and encompasses amino acids 309–433. *STK11/LKB1* has known to mediate its cellular functions through interactions with a number of proteins [20]. Although the exact function of *STK11/LKB1* remains largely unknown, studies suggest its role in cell signaling and apoptosis [21]. *STK11/LKB1* is proposed to induce cell cycle arrest through p21, be involved in p53-dependent apoptosis pathways and vascular endothelial growth factor (VEGF) signaling, as well as in BRG1-dependent chromatin remodeling and growth arrest [18, 22–24]. *STK11/LKB1* is also known to have effects on metabolism, polarity, and proliferation by phosphorylating and activating the AMPK and AMPK-related kinases [25].

In this study, we identified a point mutation C.217T > A in exon 1 of *STK11* gene, which resulted in a cysteine to serine substitution at the 73rd codon. This de novo mutation located in the catalytic kinase domain, encoded by the codons 49–309. We enquired about *STK11* homology from GenBank, and found that the nucleotide/amino acid identities in *Homo sapiens* (human), *Pan troglodytes* (chimpanzee), *Canis lupus familiaris* (dog), *Bos taurus* (cattle), *Mus musculus* (house mouse), *Rattus norvegicus* (Norway rat), *Gallus gallus* (chicken), *Danio rerio* (zebrafish), and *Drosophila melanogaster* (fruit fly) were 99.2/99.3, 89.6/93.5, 84.0/87.7, 85.2/91.0, 85.5/91.4, 80.2/90.5, 78.1/83.1, and 61.3/54.7, respectively. The 73rd amino acids residue is cysteine, which is conserved absolutely among the above-mentioned species, as shown in Fig. 4. We speculated that the conserved amino acid residue is important to the enzyme and that this missense mutation may defect the catalytic activity of the enzyme.

In conclusion, we identified a de novo mutation of *STK11* in a Chinese patient with PJS and enlarged the spectrum of mutations of the *STK11* gene. Because of the increased risk of multi-organ cancers in PJS patients [2], molecular diagnosis will be an important factor in genetic counseling, prenatal diagnosis, clinical management of patients, and tumor screening.

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